

Spontaneous blinking behaviour in persistent vegetative and minimally conscious states: Relationships with evolution and outcome

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Abstract

There is evidence that spontaneous blinking correlates with cognitive functions. This arises from the observation that blinking rate (BR) is modulated by arousal levels, basic cognitive processes (e.g., attention, information processing, memory, etc.) and more complex cognitive functions (e.g., reading, speaking, etc.). The aim of this work was to test the role of BR evaluation in the assessment of cognitive network functioning in awake patients with consciousness deficits.

Thirteen patients were recruited for the study, and were assessed by the Glasgow coma scale (GCS) and Glasgow outcome scale (GOS) on admittance and discharge, respectively. A level of cognitive functioning scale (LCFS) score was assigned at every change in awareness or at least every 2 weeks. At the same time as the clinical tests, the BR was observed for a 5-min period. Ten healthy subjects, observed throughout three non-consecutive days, formed the control group. The BR underwent a different temporal behaviour in the two diagnostic categories. In the persistent vegetative state (PVS) group it remained stable throughout time and linked with the clinical conditions of the patients; whereas in the non-persistent vegetative state (NPVS) group it decreased over time as the cognitive conditions improved. Moreover, a strong inverse correlation was found between overall BR values and LCFS scores. We have concluded that the blinking behaviour changes manifested in PVS and NPVS patients reflect different evolution phases of a cholinergic–dopaminergic imbalance, and that a reduced BR characterizes the early stages of consciousness recovery.

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1. Introduction

As a result of the great progress made in intensive care and neurosurgical techniques, it is now possible to keep many patients alive after even very serious brain damage. In fact, the rate of survival of patients in coma for over 6 h following serious head injury is approximately 50% [5,20]. Within a month of the injury, 10% remain in a persistent vegetative state (PVS). Of these, about 36% manifest a certain degree of recovery during

the following 11 months, while 11% remain in this state a year after the injury [6].

However, few possibilities exist for a direct action upon these more or less prolonged vegetative (VS) or minimally conscious states (MCS). In fact, with the exception of the occasional anecdotal case, recovery from these clinical conditions is still more often or not linked to a spontaneous evolution of the circumstances. On the contrary, management of these patients should be as pragmatic, active and motivated as possible. The decision of whether and how to start and/or sustain possible recovery with specific measures, or whether to direct efforts towards simple maintenance-support of the patient, should be guided by both a thorough knowledge of the injury suffered (from anatomical,

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histological, physiopathological and neurochemical points of view) and by the aspects of residual function in the individual case [39].

To date, numerous attempts have been made to achieve the most realistic and reliable possible prognosis regarding recovery of consciousness, both from clinical [18] and laboratory-instrumental points of view [12,14,21,24,29,30,36,40,50].

Our approach to the problem is based on previous reports concerning analyses of spontaneous blink rate (BR) in relation to the functional status of certain cognitive and/or neurotransmitter networks, both in normal subjects and in patients with central nervous system (CNS) disorders.

BR in normal subjects appears to be diversely influenced by vigilance [3], perceptive aspects [47,52], basic cognitive processes (such as attention [45], recognition or discrimination [16,48], information processing [15,16], memory [34,48]) and more complex cognitive functions (such as concentration [51], reading [4,35], conversation [4], mental calculation [42]). It can be said, in general, that it decreases during attention and information processing phases and increases during context updating and temporary memorisation applied by the working memory [15,28,35,48]. These behavioural data are supported by psychophysiological data, which imply that there is a temporal relationship between the chronometry of information processing and the rhythm of the blinks. During the first 300 ms after the presentation of a target stimulus the BR is inhibited, while it results to be facilitated during the following 500 ms [16], according to a temporal succession which calls to mind that of P300. Therefore, it is reasonable to say with Rosenbaum that BR and its variations in response to functional requirements can be considered a reflection of a person's cognitive state [38].

Moreover, BR has been studied in various diseases of the CNS, like schizophrenia, Parkinson's disease, attention deficit/hyperactivity disorder (ADHD), Tourette's syndrome [7,25,27]; diseases principally due to modifications in the dopaminergic system, in which there is an increase in BR in hyperactive states and a decrease in conditions of deficit. These facts have been confirmed by neuropharmacological studies carried out in humans and in monkeys. In particular, dopamine-agonist agents have resulted to be capable of increasing BR and dopamine-antagonists of decreasing it [25]. On the other hand, the opposite has been seen to occur in the cholinergic system: anti-cholinergic agents increase BR while cholinomimetic agents reduced it [25]. In addition, there is a certain amount of experimental evidence whereby Alzheimer patients show high BR, which inversely correlate with their attention and short-term memory capacity [32,34,43]. Hence, spontaneous blinking can be likened to an automatism, the modulation of which is the result of two prevalent and opposite forces: dopaminergic (excitatory) and cholinergic (inhibitory).

Finally, according to the neurobiological theory proposed by Crick and Koch [9–11], consciousness originates and is maintained by an automatic serial process entailing the shifting of attention, which permits interaction of an individual's sense of self with the outside world and vice versa. Despite of the discontinuous nature of this mechanism, the subjective experience of continuity of the conscious contents appears imputable

to the temporary memorisation exerted every few seconds by the working memory [22]. This latter phase might be marked or even promoted by spontaneous blinking, owing to the previously mentioned chronological similarities between blinking and information processing. On the grounds of these assumptions, and by studying the rate of spontaneous blinking, it should be possible to infer information on the functional status of cognitive networks that are vitally important for conscious functions (attention/working memory) and, consequently, in selected circumstances, on consciousness itself.

The aim of our research was to test BR as a tool for monitoring the evolution of consciousness in persistent vegetative or minimally conscious patients, and to investigate if a relationship exists between blinking behaviour and recovery of consciousness.

2. Materials and methods

All the subjects recruited for the study were observed for five consecutive minutes (hereinafter called sessions) between 11 a.m. and 12 o'clock mid-day (or rather, at a time sufficiently remote from peaks of circadian drowsiness theoretically capable of producing significant variations in blink rate), in an atmosphere of sensorial isolation and comfortable room temperature. The instant when each and every blink occurred was memorised on a digital chronometer and then recorded in a specific table (from the 1st to the 300th s) so as to preserve the pattern of temporal succession of the data. The blink rate (BR), i.e. the number of blinks within the unit of time, measured as blinks per minute (bpm), was taken into account.

A total of 13 patients were studied (nine males and four females; mean age 41.9 ± 18.5 years; range 20–73 years), with various degrees of consciousness deficits caused by cranio-encephalic injury (8), ischaemic-hypoxia (3) and hypoxic-hypoxia (2). Their state of consciousness on admission to hospital was assessed against the Glasgow coma scale (GCS) and the Rancho Los Amigos levels of cognitive functioning scale (LCFS). Assessment of state of consciousness was performed thereafter by attributing the LCFS score alone, whenever a change in the clinical and neurological status was evident (or else at least every 2 weeks) and then upon discharge. BR was assessed, in a manner that could produce homogeneous and correlated data, at the same time as these scales. The patients were supine during observation and, whenever levels of consciousness and understanding permitted, they were asked to look on front of them. The anatomical site of the lesion was detected by MRI and/or CT scan in all patients. The clinical characteristics of these patients are shown in Table 1. For analysis of the data the patients were divided into two groups, PVS [44] and non-PVS, according to whether their score in the Glasgow outcome scale (GOS) resulted to be less than or equal to or greater than, respectively, at the cut-off value of 2. This latter group included both patients in minimally conscious state (MCS) and patients who were emerging from it [19].

Three of the 13 patients examined (1 PVS, 2 NPVS) were observed three times between 11 a.m. and 12 mid-day (at intervals of approximately 20 min); this was to test the reliability and stability of the BR parameter in the test-retest at the same moment of the day. The relative data were submitted to one-way repeated measures ANOVA, but no significant differences emerged. The mean value of the three observations was therefore included in the global statistical analysis, treating it as though it were one single session.

The control group was made up of 10 normal subjects, homogeneous with the patients with respect to sex and age, recruited after excluding possible causes of conjunctival irritation (including the use of contact lens) or other ophthalmological disorders (except common dioptric complaints) and the use of psychotropic drugs. The subjects were asked to refrain from taking unnecessary mental stimulants (coffee, tea, tobacco, alcohol) during the two hours prior to the sessions. The control subjects were likewise studied in a supine position and were invited to look straight ahead, without staring at anything in particular and without paying attention to their surroundings, but to think of anything they wished. They were all assessed on three different, non-consecutive days (leaving an interval of at least 1 week between one session and another). All the experiments followed

Table 1
Synopsis of the clinical features of the patients

Case no.	Name	Sex	Age (years)	Cause	Duration of impaired consciousness (months)	GCS	LCFS	GOS	Neuroimaging	Lesion	
										Neuropathology	Location
1	Z.K.	M	48	Trauma	6	6	2	2	CT/MRI	DAI CC SDH	Corpus callosum b-Frontal lobe (polar), b-temporal lobe (polar) b-Temporo-occipital lobe, b-parietal-occipital lobe Cortical/subcortical diffuse
2	T.M.	F	35	Trauma	6	6	2	2	CT/MRI	A IVH HD A	Tri-ventricular Cortical/subcortical diffuse
3	S.F.	M	73	Haemorrhage/ ischaemic-hypoxia	2	6	2	2	MRI	ICH	r-Cerebral peduncle, r-thalamus, r-basal ganglia, r-internal capsule, r-corona radiata
4	M.A.	M	28	Hypoxic-hypoxia	72	8	2	2	MRI	IBD	r-Temporo-occipital lobe, r-fronto-parietal lobe, r-insula
5	Ce.Co.	F	55	Ischaemic-hypoxia	7	9	2	2	MRI	A	Cortical/subcortical diffuse
6	D.L.	M	60	Hypoxic-hypoxia/ ischaemic-hypoxia	7	6	2	2	MRI	IBD A	b-Thalamus Cortical diffuse
7	R.A.	F	66	Ischaemic-hypoxia	1	14	5	3	CT/MRI	IBD IBD	r-Frontal lobe, l-occipital lobe l-Midbrain (paramedian), b-thalamus, b-corona radiata, b-centrum semiovale
8	Co.Cr.	M	28	Trauma	2	13	5 → 6	5	CT/MRI	A DAI	Cortical diffuse Corpus callosum, b-pons, b-midbrain
9	B.M.	M	27	Trauma	1	11	5 → 7	5	CT/MRI	CC DAI	r-Fronto-parietal lobe Pons (central), b-pons (paramedian), r-cerebral peduncle, l-insula, l-basal ganglia, b-fronto-parietal lobe, l-centrum semiovale
10	B.E.	M	57	Trauma	2	10	2 → 4	3	CT	CC SDH HG	l-Fronto-parietal lobe l-Cerebral emisphere b-Frontal lobe (polar)
11	H.M.	M	20	Trauma	2	9	2 → 7	3	CT	CC	r-Temporal lobe (polar) b-Frontal lobe
12	M.I.	F	24	Trauma	6	9	3 → 5	3	CT	CC SDH	r-Fronto-parietal lobe, r-temporal lobe, l-parietal lobe
13	F.G.	M	24	Trauma	2	11	3 → 5	3	CT	CC CO SDH	l-Frontal lobe Cortical/subcortical diffuse r-Fronto-parietal lobe, r-temporal lobe
										CC IBD	b-Frontal lobe (basal), r-temporal lobe (polar) r-Thalamus, r-corona radiata

DAI=diffuse axonal injury; CC=cortical contusion; SDH=subdural haematoma; A=atrophy; IVH=intraventricular haemorrhage; HD=hydrocephalus; ICH=intracerebral haematoma; IBD=ischaemic brain damage; HG=hygroma; CO=cerebral oedema; b=bilateral; r=right; l=left; GCS=score on admission; LCFS=in case of recovery of consciousness, both the baseline score and that detected upon discharge were reported; GOS=score upon discharge.

the tenets of the declaration of Helsinki. Informed consent was obtained after the aims and the experimental techniques were fully explained. The experiments had the approval of the local ethical committee.

3. Statistical analysis

Before performing the statistical analysis, the BR data were normalised by means of logarithmic transformation; this proce-

dure also allowed a more linear temporal behaviour and a more homogeneous variance between the groups. Analysis of the correlation between the variables of log BR, time from admission to hospital, diagnostic category and LCFS score was carried out by means of repeated measures multivariate regression. Sex and age of the patients were not taken into consideration in the correlation variables because other authors have already demonstrated that these have no influence on blink rate [26,4]. Moreover, time

from onset was not considered either, due to the great variability in this respect; the time lapsing from the moment of admission was considered to be more appropriate for standardising the starting conditions.

At some point during the clinical course of their disorder, all the patients in the NPVS group went through a phase of documented vegetative state from which they recovered either before admission to hospital or during the period of observation. Hence, as far as a correlation between log BR and LCFS score is concerned, combining the data with those of the PVS appeared justifiable because both of these diagnostic categories represent different moments of evolution of the same pathophysiological process which, from a clinical point of view, is clearly described by the levels in the LCFS. These levels are in fact a *continuum* of behavioural reactions (cognitive performances, in a broad sense) that range from “unresponsiveness” (score 1), to “almost normal” (score 8), with other intermediate levels such as VS (score 2) and MCS (score 3, 4).

Analysis of BR inter-test variability both in patients (3 out of 13) and in the controls (10 out of 10) was performed by one-way repeated measures ANOVA. Analysis of the correlation between the BR-in, BR-out, LCF-in, LCF-out, GCS and GOS variables was carried out by Spearman's Rank Order Correlation since the data were not normally distributed. All analyses were run with the STATA 8.0 SE statistical packages. Values of $P \leq 0.05$ were considered statistically significant.

4. Results

Despite the fact that the group of patients was a small one, the number of observations (both as a whole and for each diagnostic category) was high enough to allow reliable results from a statistical point of view.

Fig. 1 gives a clear description of the different temporal behaviour of the log BR in the two groups of patients. At the origin of the two lines of regression there is a non-significant difference ($Z=1.74$; $P>0.08$) of 0.317 (BR=1.37) between

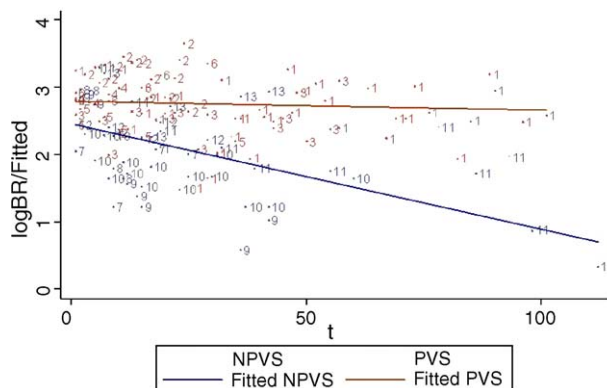


Fig. 1. Temporal behaviour of log BR in the two groups of patients. The abscissa gives the duration of hospitalisation in days and the ordinate shows the log BR of each session. The corresponding case number is shown beside each value (dots). In addition, the two regression lines are also shown: upper line (PVS), lower line (NPVS). Note how the lines progressively detach from one another, due to the steeper slope of the NPVS group. See text for further details.

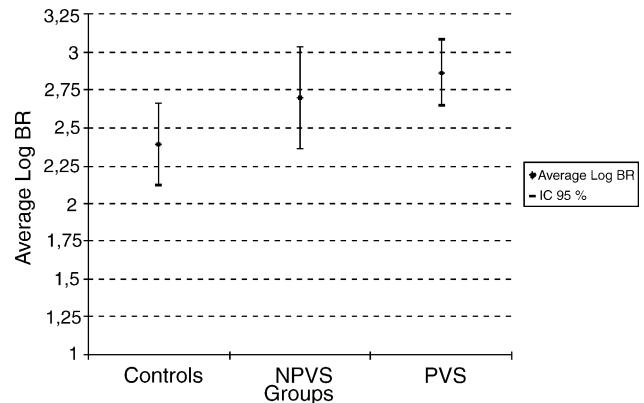


Fig. 2. Mean log BR (± 2 S.E.M.) of the two groups of patients (PVS and NPVS) in the first 7 days of hospitalisation compared with the log BR (± 2 S.E.M.) of the control group. Both groups have higher values than the controls, with a significant difference ($P=0.009$) only in the case of the PVS group. See text for further details.

the groups. After that, at every 1-day increase in the stay in hospital the difference between the two groups increases significantly ($Z=5.50$; $P>0.001$) by 0.01456 (BR=1.02) because of the steeper line of regression in the NPVS group. In fact, while the log BR behaviour in the PVS subjects is almost constant throughout time, the behaviour in the NPVS subjects drops to the extent that at each further day in hospital it decreases significantly ($Z=6.03$; $P>0.001$) by 0.0157 (BR=0.98).

This was also confirmed by log BR analysis on both the initial period (first 7 days) and on the entire stay in hospital, by comparing the two groups of patients with the controls (category variables). At the beginning of hospitalisation (Fig. 2) both groups of patients showed a higher log BR than the controls, with a difference that reached statistical significance only in the PVS group ($Z=2.63$; $P=0.009$). On the other hand, when the whole period of hospitalisation was considered (Fig. 3), the log BR of the PVS group was practically unvaried when compared with that at the start (2.75 versus 2.86) but remained significantly higher ($Z=2.09$; $P=0.037$) than that of the controls (2.75 versus 2.39). In contrast, the log BR in the NPVS

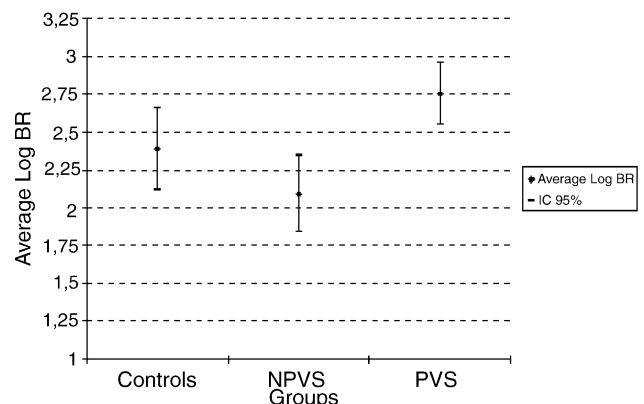


Fig. 3. The same situation as in Fig. 2 but relative to the whole period of hospitalisation. Note how the values of the NPVS group are considerably lower than at the beginning of the study and significantly lower ($P<0.001$) than those in the PVS group. See text for further details.

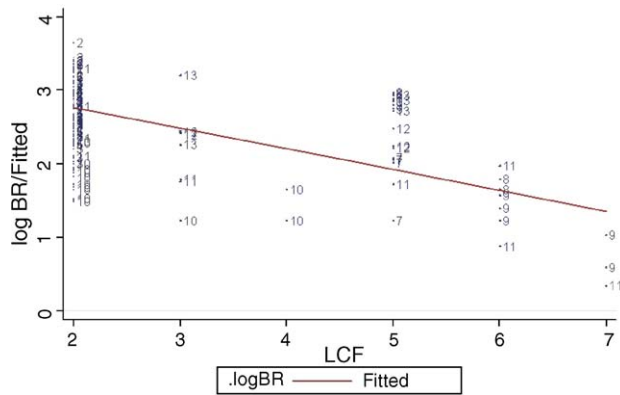


Fig. 4. The log BR values referring to the entire number of patients has been plotted against the corresponding LCFS scores. These parameters are linked together by a strong inverse correlation ($P < 0.001$). See text for further details.

group was considerably lower than the basal value (2.09 versus 2.70) with approximately 46% less than the corresponding BR (8.1 versus 14.9), and became significantly lower ($Z = 3.99$; $P < 0.001$) than that in the PVS group (2.09 versus 2.75). This is evidently attributable to the great reduction in blink rate throughout the course of hospitalisation and demonstrates that blinking behaviour in patients at different stages of the apallic syndrome is absolutely antithetic.

Moreover, Fig. 4 shows that there is a strong inverse correlation between log BR and LCFS score. In fact, the log BR tends to decrease as the LCFS levels vary, to the extent that each increase in LCFS score corresponds to a significant ($Z = -4.78$; $P < 0.001$) decrease of 0.2846 ($BR = 0.75$). This confirms that blink rate progressively decreases during the early stages of cognitive improvement in the patients.

In addition, there was a strong inverse correlation between the BR values at the moment of their discharge and the GOS scores ($R = -0.737$, $P = 0.004$), which attests the link between fewer spontaneous blinks and clinical improvement. Nevertheless, the outcome (or GOS score) could be predicted only on the basis of the GCS ($R = 0.852$; $P < 0.0002$) and the LCFS scores ($R = 0.807$; $P < 0.0008$) attributed on admission, and not on the single BR values recorded on arrival ($R = -0.040$; $P = 0.896$).

Finally, as attended, the inter-test variability of the BR in the control group was not statistically significant. This proves that in normal subjects, when the experimental conditions are kept constant, their blinking behaviour remains practically stable, even though it naturally maintains its capability to vary in response to the functional requirements.

5. Discussion

This study shows that the spontaneous BR underwent a different temporal behaviour in the two diagnostic categories. In the PVS group it remained practically stable throughout time and linked with the clinical conditions of the patients; in the NPVS group it appeared to decrease over time as the clinical conditions improved. This finds confirmation in the strong inverse correlation between the overall log BR val-

ues and overall LCFS scores, which attests the link between fewer spontaneous blinks and recovery of consciousness in the patients.

Moreover, as far as a possible prognostic role of BR testing is concerned, the single BR recordings taken at the moment of admission to the hospital resulted to be of no value. Nevertheless, the fact that the mean BR of a patient during the first week of examination is within the normal range might be of positive prognostic significance and, vice versa, negative if above the normal range. However, an indication of prognosis might be preferably obtained in an analysis of the slope of the BR/time curve beyond the first week of observation.

It has already been said in the introduction that spontaneous BR is the result of a balance between two opposite forces: dopaminergic (excitatory) and cholinergic (inhibitory). In this respect, from a theoretical point of view, an increase in blink rate could be the product of hyperactivity in the dopaminergic system (facilitation) and/or of hypoactivity in the cholinergic one (disinhibition), whereas a decrease in the rate could be the product of diminished dopaminergic activity (dysfacilitation) and/or of an increased cholinergic activity (inhibition). By comparing the results obtained with these situations of imbalance between the two systems capable of influencing BR, it is possible to outline a theoretic model of the dysfunction that underlies the altered states of consciousness being dealt with in this study. Our data show how it is possible to distinguish the natural clinical course of VS into two stages: the first stage is the lowest level of consciousness in the patient, corresponding to a high blink rate from a behavioural point of view; the second stage is the initial recovery of conscious functions, which is manifest in a decrease in blink rate. The first stage (high blink rate) might coincide with a reduction or suppression of the ascending cholinergic drive originating in the brainstem nuclei or basal forebrain. The second one (low rate) might coincide with recovered functions in the cholinergic system, which results in tonic inhibition, due to the temporary or permanent impossibility of the dopaminergic system to exert equilibration.

The available data did not allow us to found our conclusions on direct pathological correlations because of three main reasons: (i) dividing the patients into subgroups according to common sites of lesion led to having a number of observations per subgroup that was far too limited for achieving conclusions that could be considered reliable from a statistical point of view; (ii) not all patients could undergo MRI, which is more sensitive than CT scan in the case of macroscopic lesions in the brainstem, and lastly (iii) it is well known that there is frequently a lack of correspondence between clinical data and MRI when microscopic and/or metabolic damage is involved. Nevertheless, the model proposed appears to fit in with the pattern of the pathological sites most frequently found in the two main pathogenetic conditions studied: post-traumatic encephalopathy and post-anoxic encephalopathy.

A recent study [24] has reported that the presence of MRI-detectable lesions in the upper dorsolateral brainstem (the site of pedunculopontine and dorsolateral tegmental cholinergic nuclei), in patients in PVS due to trauma, coincides with a negative prognosis. Likewise, the significant reduction in the

phasic activity of REM sleep seen in this type of patient confirms the presence of damage to the cholinergic mechanisms of the pedunculopontine tegmental nucleus [33]. In this respect, it is interesting to note how only macroscopic and irreversible lesions due to III degree diffuse axonal injury (DAI) have been shown to localise in this site in 62% of the cases in the study performed by Adams et al. [1]. Moreover, this phenomenon is certainly underestimated in clinical routine. In fact, less severe degrees of DAI in this site are very likely to go undetected with MRI and consequently remain undiagnosed. Nevertheless, these microscopic injuries are capable to cause functional deficits in the cholinergic system, even if potentially reversible.

While microscopic brainstem injuries are reported in 50% of the cases, even in patients with post-anoxic encephalopathy [1,49], they are not the most important characteristic. Vice versa, injuries in the hippocampus, frontal and parietal cortex, thalamus, basal ganglia and cerebellum, make up the most typical pattern [2,49]. Similar sites are compatible with dissociation of the cortico-thalamo-cortical circuits and of the intra- and inter-hemispheric connection systems, which are on their own sufficient for accounting for even the most serious disruption of consciousness. Nonetheless, they do not offer an equally smooth-running explanation (in neurochemical terms) that can also take into account the variations seen in blink behaviour. In cases of post-anoxic encephalopathy, the cholinergic system might be involved in the basal forebrain rather than in upper brainstem level, with direct contribution of the source nuclei and/or of projections of the basalo-cortical system.

However, a selective injury of the cholinergic system without the simultaneous participation of the dopaminergic one is somewhat unlikely in both conditions. In fact, in post-traumatic encephalopathy the damage tends to be more frequently concentrated in the superior mesencephalon [1], where the source nuclei of both systems (dorsal and dorsolateral tegmentum: cholinergic; ventral tegmentum; dopaminergic) are anatomically very close to one another, since it can lead to a possible interruption in their ascending drive. On the other hand, involvement of the basal ganglia and thalamus is a frequent event in post-anoxic encephalopathy [2,49], so the striato-thalamo-cortical (prefrontal dorsolateral) dopaminergic circuits, which appear to have a vital role in controlling executive functions and working memory [13,23,37,41], might also be disconnected. Moreover, the striatum is considered the elective site where the complex reciprocal interactions between the dopaminergic and the cholinergic system take place [17,37,46]. In particular, certain data support the importance of the cholinergic system in modulation of the striatal dopaminergic activity [17,46]. Hence, a direct injury here, or a reduction in the ascending cholinergic drive, might lead to neurotransmitter imbalance, with repercussions on the prefrontal dorsolateral cortex activity by means of the self-same striato-thalamo-cortical circuits.

It is plausible to say, then, that both systems cooperate in consciousness, but only according to a precise hierarchic rule: the cholinergic system more likely performs tasks that are essential for maintaining states of arousal (by exerting cortical bioelectric

activity) and is involved in low-profile attention requirements like automatic orientation [8]; the dopaminergic system, on the other hand, has a more 'executive' role in attention functions, like attentional set-shifting or working memory [8], which are certainly more pertinent to the awareness concept, but are implausible without cholinergic 'facilitation'.

The two stages distinguished on the grounds of spontaneous blinking behaviour (corresponding to the vegetative state and to the early stages of recovery from it) suggest hypoactivity of the cholinergic system followed by its functional recovery, which in the beginning has yet to be counterbalanced by adequate dopaminergic activity. If this were true, then it remains to understand why the dopaminergic system is slower in recovering. Even if the involvement of both systems were to be acknowledged, the cholinergic recovery phase could be assumed to be the key requirement for the subsequent dopaminergic recovery to take place. A sufficiently lengthy period of cholinergic inactivity might induce phenomena of long-term adaptation in the functional arrangement of the striatal dopaminergic receptors (e.g., a down-regulation), of which the new and inverse tendency to reinstate the cholinergic drive might involve a certain degree of latency. A possible alternative might be lie in a slower regeneration of the meso-cortical dopaminergic projection pathway (monosynaptic) where, although the distance to be recovered is the same, the regenerating segment is longer than the pontomesencephalo-thalamo-cortical cholinergic pathway (multisynaptic) [31].

Some considerations have yet to be made which lead us to be somewhat cautious in drawing definite conclusions. Firstly, it is necessary to stress that the BR is not a direct biological index but rather an indirect behavioural one, since it can indicate a level of underlying activity by means of a behavioural parameter. Nevertheless, its connection with certain cognitive (even basal) and/or neuro-transmittal functions is extensively reported in the literature. Secondly, a comparison between patients and control subjects might produce some uncertainties because of the probable cognitive implications of BR in the latter, even at rest. However, while the BR of the control subjects did not vary in the conditions kept constant throughout the study, the important point in our study is that the BR of the patients decreased as their cognitive conditions improved. Lastly, the results obtained will certainly require further confirmation from studies performed in a larger population.

However, the results of this pilot study seem sufficiently encouraging to suggest further works on the subject, in particular a search for correlations between BR and biological-functional indices (e.g., by means of pre- and postsynaptic receptor imaging) and experimental confirmation of the pathogenetic model of neurotransmitter unbalance proposed herein (e.g., using neuromodulating drugs).

To conclude, analysis of spontaneous blinking behaviour is a promising tool for investigating the functional condition of the cognitive networks and the neuro-anatomical and neurochemical systems that underlie conscious functions in patients in, or coming out of, PVS. This might offer new elements for understanding the pathophysiological mechanisms responsible for altering consciousness and the relative recovery.

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